

Needle sclerenchyma variation correlates with temperature and precipitation across Atlas cedar populations in Morocco

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ABSTRACT

Leaf morphology is highly responsive to environmental conditions and plays an important role in plant adaptation along climatic gradients. However, anatomical variation in needle sclerenchyma cells has rarely been examined across populations of *Cedrus atlantica*. This study, investigates variations in the sclerenchyma cells of needles across three Moroccan Middle Atlas cedar populations: two populations from Kha and Mou in Azrou region, and one population from Tam in Ifrane region, at an altitude of between 1.605 and 1.823 meters. Several sclerenchyma cell traits in needle cross-sections were measured: Number and area of sclerenchyma cells at the hypoderm the endoderm and surrounding the resin canal; number of sclerenchyma cell: the type with thick walls and restricted lumen, type with intermediate character and cell type with thin wall and wide lumen in the endoderm and in the surrounding resin canal, and their variation among populations was analysed using a light numeric microscope (Optika DM-15) with ten trees of each population and ten needles per tree. Descriptive statistics, a Student's t-test, discrimination power, and a dendrogram based on the closest Euclidean distances were calculated for the traits. The descriptive statistics revealed variation in the area of hypodermal sclerenchyma cells, which varies among populations. A Student's t-test of the mean percentage of the different types of sclerenchymatic cells in the endodermis and surrounding the resin canals revealed significant differences between the analysed cedar populations. In contrast, agglomeration of populations over short Euclidean distances revealed the sclerenchyma cell traits consistently separated two major population groups corresponding to cold subhumid and fresh humid climatic conditions (Climate-Data.org.). Analysis of the discrimination power revealed a high level of distinction between the studied populations of *C. atlantica* for four of the twelve analysed needle traits. These results suggest that needle sclerenchyma traits reflect ecological differentiation associated with temperature ($T= 11.3\text{ }^{\circ}\text{C}$ for Tam and $14.2\text{ }^{\circ}\text{C}$ for Kha and Mou) and precipitation ($P= 843\text{ mm}$ for Tam and 779 mm for Kha and Mou) regimes. These traits may provide useful anatomical markers for studying and conserving *Cedrus atlantica* at a population level in Morocco.

Keywords: Altitude, anatomical traits, *Cedrus atlantica*, needle, population, sclerenchyma cell.

INTRODUCTION

The leaf is an important functional organ in plants and the variation inits the diversity in plant morphology has long served as a fundamental agent in developmental and evolutionary processes (Ye et al. 2020). Morphological and anatomical studies have shown that the Sclerenchyma cells (CS), play a major role in providing

mechanical strength to plants, and their location in the central needle region has been found to justify this function while allowing needle bending (Zhu et al. 2017, Weng et al. 2020, Jankowski et al. 2021). These variations have always been attributed to stress tolerance and have been found to correlate with environmental factor gradients. They are also found in three main locations within the needle: a single-cell layer around the resin

canal, strands positioned among and above vascular bundles, and layers within the hypodermis in *P. sylvestris*, *P. uncinata*, *P. uliginosa*, *P. rotundata*, *P. mugo* and *P. omorika* (Farjon, 1984; Hejniewicz, 1993, Pushalska et al. 2006) preventing needle collapse when the needles are dehydrated or frost-damaged (Anonymous). Furthermore, Grill et al. (2013) demonstrated that the sclerenchymatic tissues of the hypodermis and those adjacent to the vascular bundles increased in the needles of 5-year-old *P. canariensis* seedlings stressed by drought due to a significant increase in the number, not size, of sclerenchymatic cells. Furthermore, Boratyńska et al. (2008) demonstrated that age affects the frequency of sclerenchyma cell types (thick-walled vs. thin-walled) in the vascular bundle gaps and resin canals of *P. uliginosa* and *P. sylvestris* needles.

In other studies, the arrangement of sclerenchyma cells (around the resin canals and between the vascular bundles) and the spacing of these bundles are key taxonomic criteria for identifying pine hybrids (Szweykowski 1969, Keng et al. 1961, Bobowicz 1990, Sobierajska and Boratyńska 2008). For example, the vascular bundle phloem of *Pinus sylvestris* and *P. uncinata* has stronger abaxial sclerenchyma than that of *P. mugo*, which has weaker development (Boratyńska and Boratyński 2007). *Pinus sylvestris* primarily exhibits fiber-like sclerenchyma with thick walls and a restricted lumen, whereas in *P. mugo* predominantly exhibits cells with slightly thickened walls and a distinct lumen.

Other research analyzed the structural and anatomical characteristics of fully developed, intact needles from the sunlit crown of ten to thirty healthy trees, with ten old needles representing each individual tree from nine *Cedrus* populations: Distributed along an altitudinal gradient of 1100–1750 m, *C. atlantica* inhabits the Rif and Middle Atlas, *C. brevifolia* is found in the mountains of Cyprus, and *C. libani* occupies the Taurus, Antitaurus, and Lebanon mountains. The researchers found significant differences in the number of sclerenchyma cells in the endodermis between the studied populations, as well as between populations within species in terms of the number of sclerenchyma cells in the endodermis and the percentage of cells around resin canal types (Jasińska et al., 2013). Conversely, the variation of sclerenchyma cell traits at the hypodermis, endodermis and resin canal levels in natural populations of *C. atlantica* has never

been studied, despite these traits being considered important taxonomic characteristics that vary with environmental factors (Boratyńska and Boratyński, 2007; Boratyńska et al., 2008; Sobierajska and Boratyńska, 2008).

Unfortunately, the *Cedrus atlantica*, which is endemic to the Atlas Mountains, has been listed as an endangered species by the International Union for Conservation of Nature due to a sharp decline in its population over the last 40 years (Laala and Adimi, 2024). These authors, modelled the current distribution of *C. atlantica* in Algeria using 103 sites, and projected the impact of climate change on its distribution in the future (2050–2090). This study found that altitude and annual temperature are the key factors influencing the presence of the Atlas cedar in Algeria. The results also show that the species is particularly vulnerable to climate risks in low-lying areas and in regions further south. Similarly, Chaddadi et al. (2017) carried out a transient model simulation for the period 1960–2010 on cedar populations in Morocco. This simulation revealed that the distribution of Atlas cedar has declined by roughly 75% over the past five decades. It also indicated that eastern populations in the Rif Mountains are more severely threatened by limited water availability than their western counterparts, and that these populations now survive only in small, isolated areas such as Jbel Kelti, Talassemtane, Jbel Tiziren, Oursane, and Tidighine. This persistence is supported by the presence of microclimates shaped by moisture influences from the Atlantic Ocean and the Mediterranean Sea. According to Rhanem (2011), the Atlas cedar is declining in arid areas, forcing its lower distribution limit to shift to higher altitudes in the Middle Atlas. Sarmiento (2011) recently noted that UNESCO's designation of Morocco mountain landscapes as essential for conservation supports the inclusion of the *Cedrus atlantica* Biosphere Reserve. Consequently, it is vital to investigate the mechanisms allowing species like *Cedrus atlantica* to endure significant historic climate changes in the Moroccan mountains. Generally, plant species adapt to climate change by adjusting their range and/or undergoing physiological and genetic changes (Tierney et al., 2017). Moreover, as mentioned above, the role of sclerenchyma cells in leaf adaptation has been extensively studied (Grill et al., 2004; Germain et al., 2014; Sun et al., 2015; Adame-González et al., 2019). Additionally, several evaluations of stand variability in terms of needle morphology and

herbivory have been conducted on various coniferous species (Germain et al., 2014). However, significant gaps in our knowledge of the role of sclerenchyma cells in *C. atlantica* remain, despite the disappearance of more *C. atlantica* populations due to structural damage to the needles and global warming induced by climate change in North Africa (Germain et al., 2014; Bouahmed et al., 2019).

The main objective of this study was to evaluate the variation in the needle sclerenchyma cells of three natural populations of *Cedrus atlantica*: two in the Azrou region (Kha and Mou) and one in the Ifrane region (Tam). The populations were sampled over an altitudinal range of 1605–1823 m in Morocco. Specifically, we aimed to determine whether:

- sclerenchyma cell traits at the hypodermis, endodermis, and resin canal levels vary significantly among populations, by using quantitative analysis;
- these anatomical traits can reliably discriminate populations along altitudinal and climatic gradients (Table 1, Figure 3).
- sclerenchyma variation reflects adaptation to environmental stressors associated with Moroccan climate change [1, 2].

This study aims to address the current knowledge gap concerning anatomical adaptation in *C. atlantica* and to provide new insight into population differentiation and potential conservation strategies.

MATERIALS AND METHODS

Sampling and measurements

The field materials were sourced from the Azrou and Ifrane regions of the Moroccan Middle Atlas in spring 2014 (April–May). A total of 10 mature whorls per individual were collected from 10 individuals in each of the following natural populations of *Cedrus atlantica*: Moudemame (Mou), Kharzouza (Kha) in the Azrou region, and Tamrabta (Tam) in the Ifrane region (Table 1). Research indicates a genetic divergence between the Kha and Tam populations (Terrab et al., 2008), whereas the Mou population has never been genetically studied. The mean annual temperature and precipitation data for each population location (Table 1 and Figure 2) were sourced from climate-data.org [1, 2]. Plant material was collected from ten healthy, mature trees in each population, with individuals spaced at least 30 m apart (Table 1).

From each tree, ten fully developed, undamaged, mature whorls that had formed under similar light conditions were sampled. The material was preserved in 70% alcohol and stored at -20°C before sectioning for up to a month without observation of damage. We sampled 10 needles from 10 trees per population, selecting one mature needle (oldest/longest) from each whorl for analysis. Free-hand cross-sections were cut from the middle of each needle. Ten of the thinnest samples were then cleared using a 5% NaOH solution at 70°C for four hours. The section that best represented the sample was selected for cellular measurements. Tissue clearing was performed using the Arnott and Brady methods, as referenced by Ruzin (1999). After mounting the treated sections between a blade and a coverslip, they were observed under a DM-15 Optika light numeric microscope (Ponteranica, Italy) with a magnification of $\times 40$, $\times 100$ and $\times 400$. The anatomical preparations were then photographed, and the images were saved for analysis (Figure 2). A total of 100 needles per population were used for the analyses.

Biometric analysis of twelve characteristics was carried out using the Opmias software (version 1.3.0.0), with a resolution of $1\ \mu\text{m}$. For each individual, ten needles were examined, with ten cross-sections taken from each, selecting the most transparent sections to ensure optimal cellular observation. It is calibrated using a calibration grid with known graduations in millimetres. First, we measure the grid in mm using the software (pixels). Second, we calculate the scale factor (distance in $\text{mm} \times 1000$)/pixels). We apply the factor to measure in μm . The total number and area of sclerenchyma cells were determined at the hypodermis, endodermis (between the vascular bundles and above the phloem), and resin canal levels. In addition, the number of sclerenchyma cell types (A, B, and C) in the endoderm and (A', B', and C') in the resin canal region were determined (Figure 1, Table 2).

Data analysis

Descriptive statistics (mean, minimum, maximum, and coefficient of variation) were calculated for each trait in the three populations (Tables 3, 4, and 5). The percentage and average frequencies of the types of sclerenchyma cells within the endoderm and at the periphery of the resin canals, by individual and by population, respectively, were determined by applying the following formula: $X/Y \times 100$, where:

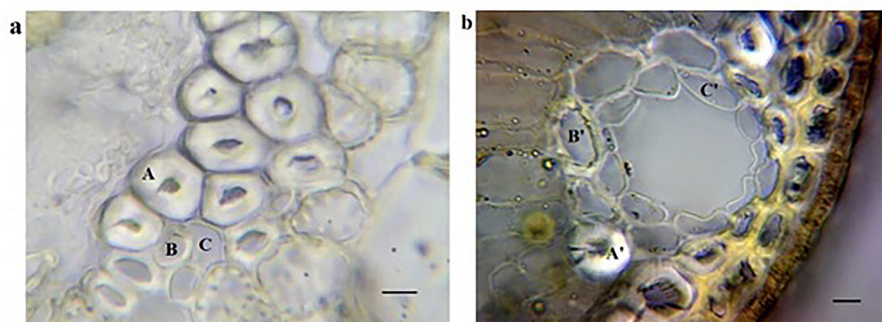


Figure 1. Types of Sclerenchyma cells in the needles of the *Cedrus atlantica* populations. a: in the endoderm. b: surrounding resin canal. A, A': sclerenchyma cells with thick walls and restricted lumen; B, B': sclerenchyma cells with intermediate character; C, C': Sclerenchyma cells with thin wall and wide lumen. The scale bar corresponds to $3 \times 10^3 \mu\text{m}$

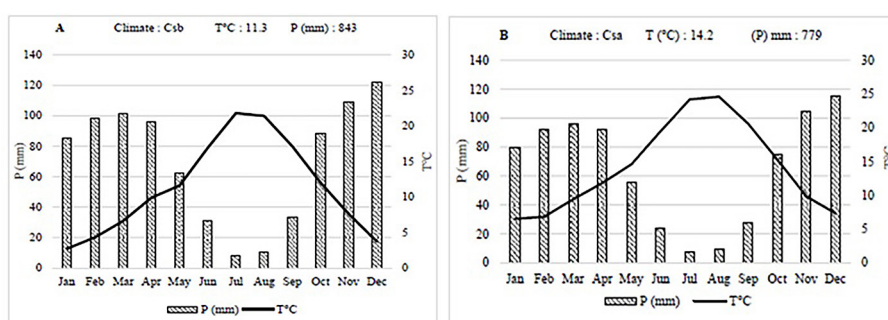


Figure 2. Climate graph, (A) of the Ifrane region (Tam) [1] and (B) of the Azrou region (Kha and Moud) [2] during the year 2012. Csa: hot-summer mediterranean climate; Csb: warm-summer mediterranean climate; Bsk: cold semiarid climate; T: annual mean temperature; P: annual precipitation

Table 1. Characteristics of three *Cedrus atlantica* populations sampled along a Middle Atlas transect in Morocco

Regions	Populations	Number of selected trees	Coordinates	Altitude [m, amsl]	Substrate
Middle Atlas Ifrane	Tamrabta (Tam): A sparse stand of maritime pine or holm oak on a plateau with a 10% slope	10	33°37'N 5°03'W	1605	Calcareous dolomite/sander
Middle Atlas Azrou	Moudemame (Mou): A homogeneous and stable population, with a zero slope.	10	33°25'N 5°11'W	1780	Basalt-calcareous
	Kharzouza (Kha): A clear stand of holm oaks on a plateau at the summit, bordered by a slope with a 37% incline	10	33°24' N 5°12' W	1823	Basalt calcareous

- by individual:
 - X = the sum of the number of each type of CS
 - Y = the sum of the number of all types of CS
- by population:
 - X = the sum of the frequencies of each type of CS of the 10 individuals
 - Y = the sum of the frequencies of all types of CS of the 10 individuals

The Shapiro–Wilk test was used to verify the distribution of the data, and the Levene test was

used to assess homoscedasticity prior to performing multivariate comparisons. A student's t-test for independent samples was used to test the statistical significance of differences in mean values of the percentages of sclerenchyma cell types among the cedar populations at a significance level of $P \leq 0.05$ (Sokal and Rohlf, 2003), without the use of multiple comparison tests.

Discrimination power was calculated for each trait at significance levels of $P \leq 0.01$ and $P \leq 0.05$

Table 2. Anatomical characteristics of needles sampled from three population groups

Abréviations	Traits
NCSH	Number of sclerenchyma cells at the hypoderm
ACSH	Area of sclerenchyma cells at the hypoderm (μm^2)
NCSE	Number of sclerenchyma cells in the endoderm
ACSE	Area of sclerenchyma cells in the endoderm (μm^2)
NCSCR	Number of sclerenchyma cells surrounding the resin canals
ACSCR	Area of sclerenchyma cells surrounding the resin canals (μm^2)
NA & NA'	Number of sclerenchyma cell type with thick walls and restricted lumen in the endoderm (A) and in the surrounding resin canal (A')
NB & NB'	Number of sclerenchyma cell type with intermediate character in the endoderm (B) and in the surrounding resin canal (B')
NC & NC'	Number of sclerenchyma cell type with thin wall and wide lumen in the endoderm (C) and in the surrounding resin canal (C')

(Sokal and Rohlf, 2003). Following a stepwise discriminant analysis of all the characters, the relationships between the populations were visualised using a graph that compared the first discriminant functions, and the Euclidean distances among populations were estimated using all characters except for number and area of sclerenchyma cells surrounding the resin canals, and agglomerations of populations based on the shortest Euclidean distances, as determined by Ward's method, were analysed to verify the relationships between populations revealed by discriminant analysis. Hierarchical analysis of variance was applied to estimate the percentage

variation of each character between populations (Sokal and Rohlf, 2003). Data processing was divided between two tools: IBM SPSS Statistics 20.0 for multivariate analyses, and Microsoft Excel for descriptive statistics and frequency percentages.

RESULTS

Characterization of the sites

The Tam forest experiences lower rainfall in the northeast compared to the southwest, a pattern

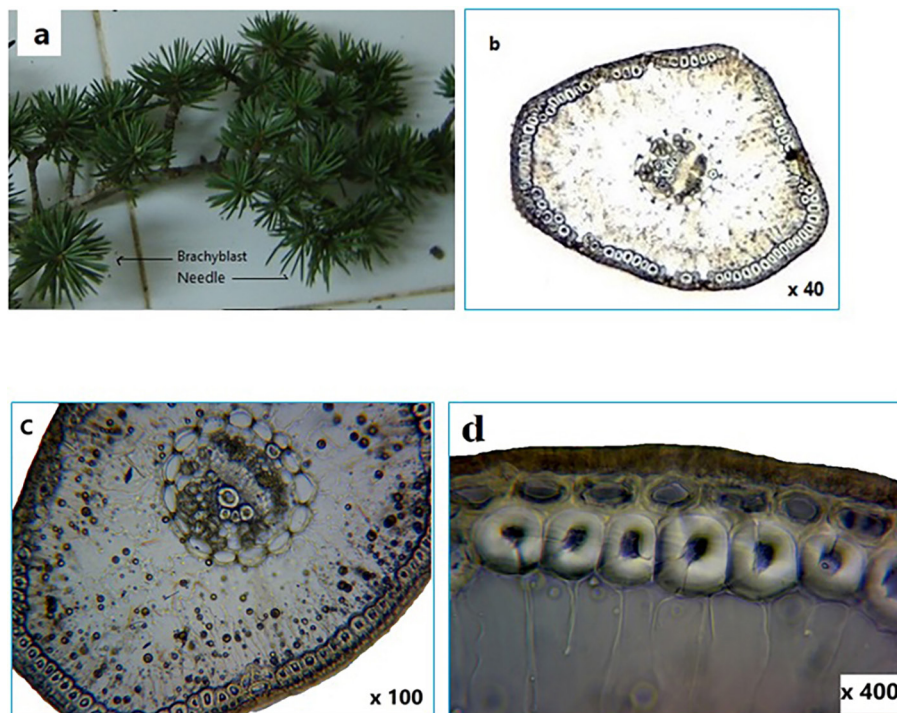


Figure 3. Sampled brachyblasts (a) and the needle cross sections viewed at different magnifications, b x 40, c x 100 and d x 400

Table 3. Statistic description of analyzed anatomical traits in the hypoderm in the needles of Moudemame (Mou), Tamrabta (Tam) and Kharzouza (Kha) populations

Populations	Statistics	NCSH	ACSH × 10 ³ μm ²
Mou	Minimum	75.00	42.50
	Maximum	138.00	195.66
	Mean	103.73	80.47
	Standard error	14.46	33.34
	Variation coefficient (CV)	13.94	41.43
Tam	Minimum	75.00	33.66
	Maximum	140.00	203.98
	Mean	105.82	79.44
	Standard error	12.76	20.11
	Variation coefficient (CV)	12.06	25.32
Kha	Minimum	79.00	45.22
	Maximum	120.00	184.66
	Mean	97.51	105.19
	Standard error	8.66	39.78
	Variation coefficient (CV)	8.88	37.81

Note: NCSH – number of sclerenchyma cells at the hypoderm; ACSH – area of sclerenchyma cells at the hypoderm.

Table 4. Descriptive statistics of anatomical traits of sclerenchyma cells in the endoderm in the needles of Moudemame (Mou), Tamrabta (Tam) and Kharzouza (Kha) populations

Populations	Statistics	Global number and area		Cell type		
		NCSE	ACSE × 10 ³ μm ²	NA	NB	NC
Mou	Minimum	3	1.12	0	1	0
	Maximum	22	17.23	17	17	9
	Mean	10.47	5.30	4.22	5.23	1.07
	Standard error	3.88	2.91	2.74	2.69	2.07
	CV	37.06	54.97	64.90	51.38	194.47
Tam	Minimum	4.00	1.53	1	0	0
	Maximum	21.00	10.92	15	10	6
	Mean	11.36	5.43	7.76	2.92	0.80
	Standard error	3.75	1.83	3.00	2.24	1.36
	CV	32.98	33.70	38.70	76.60	169.16
Kha	Minimum	3	2.35	0	0	0
	Maximum	24	18.97	15	10	5
	Mean	10.65	7.72	6.09	3.80	0.76
	Standard error	3.61	3.60	3.59	2.40	1.32
	CV	34.04	46.65	58.99	63.31	173.60

Note: NCSE – number of sclerenchyma cells in the endoderm; ACSE – area of sclerenchyma cells in the endoderm; NA – number of sclerenchyma cells with thick walls and restricted lumen; NB – number of sclerenchyma cells with intermediate character; NC – number of sclerenchyma cells with thin wall and wide lumen.

that matches the declining altitudinal gradient (Labhar et al., 2012). Unlike the Kha population, this massif is characterized by a steep slope and specific soil and climatic conditions. Compared to Mou, only the climatic and edaphic variables

mark a differ (Figure 3, Table 1). The presence of a steep slope constitutes the only structural difference between the Kha and Mou forests (Table 1). If these mountains share a population of *C. atlantica* and *Quercus ilex*, the Kha forest

is specifically characterized by the dominance of the latter species compared to Mou.

Sclerenchyma cell growth

The compared cedar populations differ from each other in terms of the growth of sclerenchyma cell needles in three locations: the hypodermis, the endodermis and around the resin canals.

At the hypoderm

The mean number of sclerenchyma cells in the hypodermis (NCSH) is low (97.51) in the Kha population, and is slightly higher in the Mou and Tam populations (103.73 and 105.82, respectively). Similarly, the average area of the hypoderm (ACSH) of Kha needles is high ($105.19 \times 10^3 \mu\text{m}^2$), whereas it is lower for Mou and Tam ($80.47 \times 10^3 \mu\text{m}^2$ and $79.44 \times 10^3 \mu\text{m}^2$ respectively). However, the NCSH of Kha needles is more stable (8.88%) than that of the other two populations (12.06% and 13.94%, respectively) (Table 3). Observation of the growth of the type of sclerenchyma cells in the three populations under study revealed that only one type of CS (cells with thick walls and a restricted lumen) exists in the hypoderm, which distinguishes between the three populations, as shown in Table 3.

At the endoderm

The mean number of sclerenchyma cells (SC) in the endodermis varied slightly (from 10.47 to

11.36) between the populations studied. However, the mean area of the CS was larger in Kha needles ($7.72 \times 10^3 \mu\text{m}^2$) than in the needles of the other populations (Figure 4, Table 4). Additionally, intra-population variation analysis revealed that the number of CS (NCSE) exhibited a similar coefficient of variation (CV) across the three populations, ranging from 32.98% to 37.06%. However, variation in cell area was more pronounced in Mou and Kha (54.9% and 46.65%, respectively) than in Tam (33.7%).

In terms of sclerenchyma cell types, the average number of cell A, which has thick walls and a restricted lumen, is important in the vascular bundles of Tam needles, with a value of 7.76. This is less important in Mou (4.22) and Kha (6.09). Conversely, the average number of cell B, which has intermediate characteristics, is higher in Mou than in the other two populations, with an average value of 5.23. Cell C, which has a thin wall and a wide lumen, is the least prevalent in the three populations, with an average value ranging from 0.74 to 1.07. These three types of cell represent strong variation between the populations, with a coefficient of variation (CV) ranging from 38.70% for cell A in Tam needles to 194.47% for cell C in Mou needles (Table 4).

The frequency of the different types of CS in the endoderm of each individual shows that types A and B are dominant in all three populations, reaching up to 82.98% at P6 in Tam for type A and 74.39% at P2 in Mou for type B. Type C is the least prevalent in all three populations (Figure

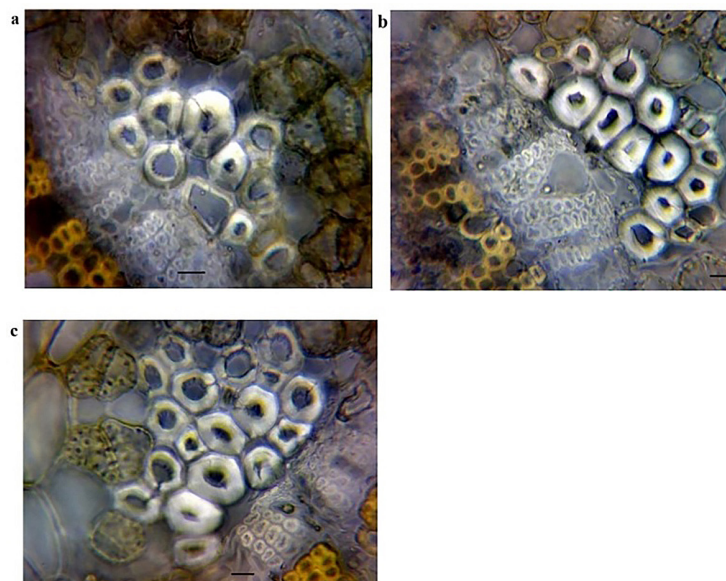


Figure 4. Sclerenchyma cells of Mou (a), Tam (b) and Kha (c) populations. The scale bar corresponds to $3 \times 10^3 \mu\text{m}$

4). The mean frequency of these three types of CS in the endoderm of the studied needles differs sharply between populations. Types A and B are the most prevalent in Mou and Kha needles, with an average frequency ranging from 37.80% to 53.32%. Tam needles, however, are characterised by type A dominance, with an average frequency of 67.80%. Conversely, type C is the least prevalent, with an average frequency of no more than 9.38% across the three populations (Figure 5).

At the surrounding the resin canals

The mean number of sclerenchyma cells around the resin canals varied slightly among the studied populations. Furthermore, the coefficient of variation for this number was higher in the Tam needles than in the other populations, at 38.60%, 34.75% and 32.88% respectively (Table 5). The average area value (ACSCR) of these cells was larger in the Tam needles at $7.75 \times 10^3 \mu\text{m}^2$. The Mou and Kha populations had smaller areas, reaching at $5.91 \times 10^3 \mu\text{m}^2$ and $5.53 \times 10^3 \mu\text{m}^2$ respectively (Table 5). The

coefficient of variation of the area values varied considerably across all three populations, ranging from 0.06% to 0.19%.

The populations under study are distinguished from each other by the average number of sclerenchyma cells types around the resin canals. The Mou and Kha populations are characterized by cell type B' dominance, with average values of 15.54 and 16.10, respectively. The Tam population is characterized by type A' with a value of 13.33 and type C' with a value of 14.76. The CV of these three cell types ranges from 37.14% to 67.55% within the studied populations, indicating reduced stability. Sclerenchyma cell type frequency around resin canals differs greatly across 30 individuals of the three populations. The Mou population is characterised by the dominance of thin-walled, wide-lumen cells (C'), ranging from 35.68% in individual P8 to 53.41% in individual P9, while the Kha population exhibits a frequency distribution belonging to intervals close to those of the Mou population. Meanwhile, the thick-walled, restricted-lumen (A') and intermediate-character (B') cell types were less prevalent in the

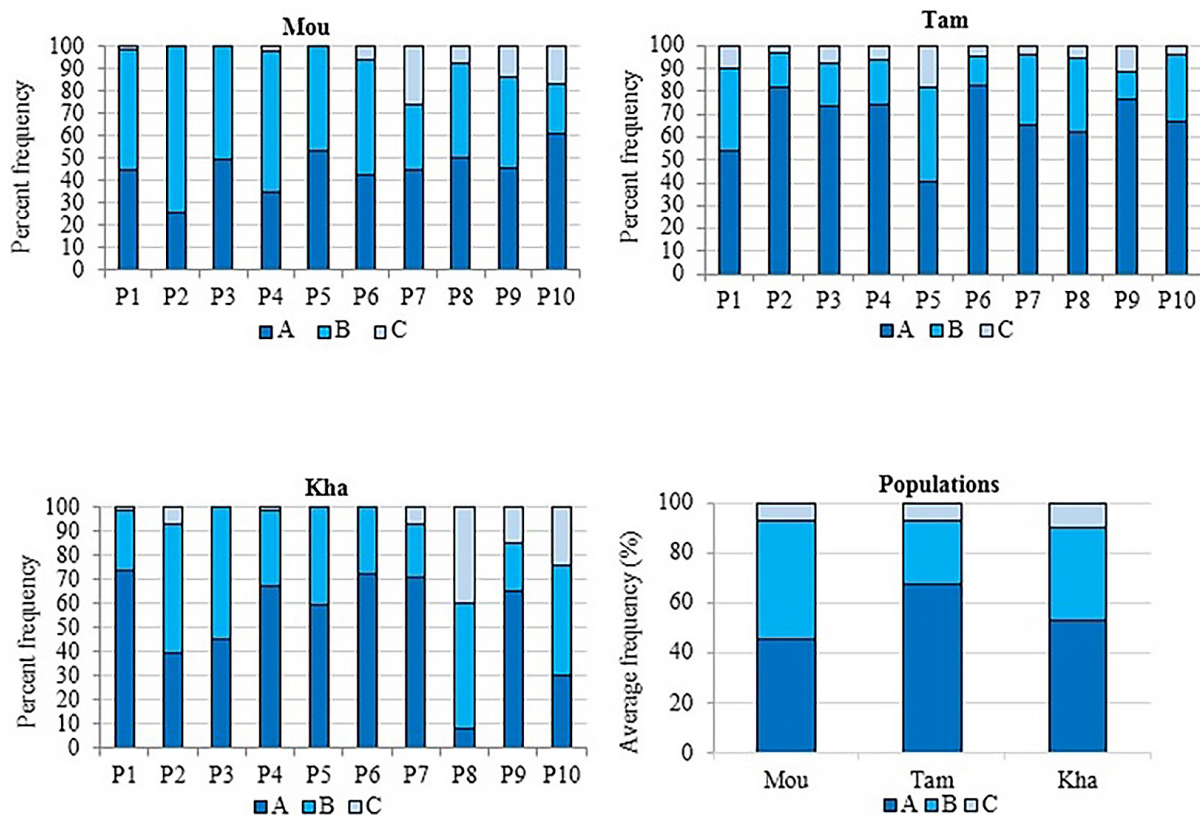


Figure 5. Variation of sclerenchyma cell types in the endoderm within population (P1 to P10) and between populations: Moudemame (Mou), Tamrabta (Tam) and Kharzouza (Kha). A: sclerenchyma cells with thick walls and restricted; B: sclerenchyma cells with intermediate character; C: sclerenchyma cells with thin wall and wide lumen

Table 5. Descriptive statistics of anatomical traits of needle sclerenchyma around the canal of resin of the Moudemame (Mou), Tamrabta (Tam) and Kharzouza (Kha) populations

Populations	Statistics	Global number and area		Cell type		
		NCSCR	ACSCR × 10 ³ μm ²	NA'	NB'	NC'
Mou	Minimum	15.00	0.19	0.00	0.00	0.00
	Maximum	60.00	86.67	26.00	25.00	38.00
	Mean	35.90	5.91	10.27	10.09	15.54
	Standard error	11.96	11.51	5.69	5.28	7.31
	CV	32.88	0.19	55.45	52.35	47.06
Tam	Minimum	13.00	0.12	3.00	0.00	0.00
	Maximum	57.00	82.34	25.00	24.00	33.00
	Mean	34.00	7.75	13.33	5.91	14.76
	Standard error	13.12	11.15	5.87	3.99	7.12
	CV	38.60	0.14	44.06	67.55	48.21
Kha	Minimum	17.00	0.39	0.00	1.00	7.00
	Maximum	58.00	11.68	27.00	28.00	30.00
	Mean	34.81	5.53	10.14	8.57	16.10
	Standard error	12.10	3.41	6.11	5.33	5.98
	CV	34.75	0.06	60.24	62.24	37.14

Note: NCSCR – number of sclerenchyma cells surrounding the resin canals; ACSCR – area of sclerenchyma cells surrounding the resin canals; NA' – number of sclerenchyma cells with thick walls and restricted lumen; NB': number of sclerenchyma cells with intermediate character; NC' – number of sclerenchyma cells with thin wall and wide lumen.

Mou and Kha populations, with frequency distributions that overlap. For type A' cells, the frequency distribution in Mou ranged from 16.28% in P2 to 39.69% in P6, and in Kha from 15.89% in P4 to 40.63% in P9. For type B' cells, the frequency distribution in Mou ranged from 16.85% in P10 to 39.93% in P7, and in Kha from 13.17% in P7 to 34.69% in P2 (Figure 6).

The average frequency of resin canal sclerenchyma cells varied between the populations studied. The Mou and Kha populations behaved similarly to each other and to the Tam population in terms of the distribution of sclerenchyma cell types (Figure 6).

Multivariate analysis of populations

Statistical analysis (Student's t-test) showed that the mean percentages of endodermal and resin canal-associated sclerenchyma cells differ significantly among the studied cedar populations. This difference was most evident in the intermediate character sclerenchyma cells (B and B') in both locations. These distinguish the Tam population from the others. Furthermore, the thick-walled, restricted lumen cells of the endoderm (type A) distinguished the Tam population from the Mou

population only. This type of cell surrounding the resin canals (A') differentiated the Tam population from the Kha and Mou populations. For cells with thin walls and a wide lumen (C and C'), differentiation between the studied populations was not significant (Figure 7).

Analysis of the discrimination power revealed that four of the 12 cedar needle traits analysed had a high level of distinction between the studied populations (Table 6). Compared to other traits, a maximum discriminating value ($P \leq 0.05$ and $P \leq 0.01$) was observed for endodermal and cells around the resin canals, specifically those of the sclerenchymal type with a restricted lumen (A, A') or of the intermediate type (B, B'). Agglomerating the Agglomerating the populations by Euclidean distance revealed that the Tam (cold and humid climate) is clearly distinct among the Mou and Kha (cool and humid climate) populations (Figure 8).

DISCUSSION

Characterization of the site

The three studied populations of *Cedrus atlantica* are located at different altitudes and

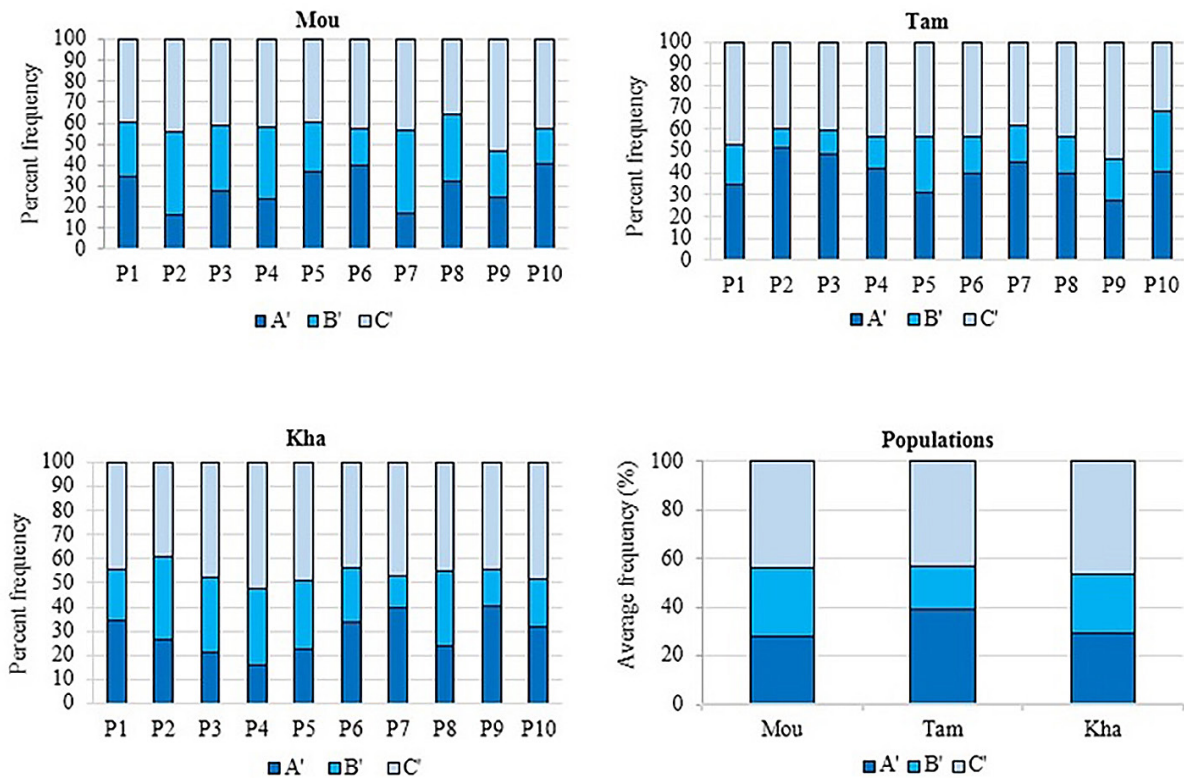


Figure 6. Variation of sclerenchyma cell types surrounding the resin canals within population (P1 to P10) and between populations: Moudemame (Mou). Tamrabta (Tam) and Kharzouza (Kha). A': sclerenchyma cells with thick walls and restricted lumen; B': sclerenchyma cells with intermediate character; C': sclerenchyma cells with thin wall and wide lumen

	A		B		C	
Mou	0.308		0.136		0.639	
Tam	0.89	0.001*	0.036*	0.008*	0.218	0.366
	<u>Kha</u>	<u>Mou</u>	<u>Kha</u>	<u>Mou</u>	<u>Kha</u>	<u>Mou</u>

	A'		B'		C'	
Mou	0.941		0.360		0.068	
Tam	0.006*	0.009*	0.026*	0.004*	0.113	0.999
	<u>Kha</u>	<u>Mou</u>	<u>Kha</u>	<u>Mou</u>	<u>Kha</u>	<u>Mou</u>

Figure 7. Results of student's t-test for three types of sclerenchyma cells in the endoderm and surrounding resin canals from all populations. A, A': Sclerenchyma cells with thick walls and restricted lumen; B, B': sclerenchyma cells with intermediate character; C, C': sclerenchyma cells with thin wall and wide lumen. *: significance at level P = 0.05

climatic conditions in the Middle Atlas of Morocco (Figure 3, Table 1). In this study, Tam, the low-altitude site, exhibited higher temperatures and lower summer precipitation with an irregular rainfall distribution throughout the year compared to the high-altitude sites Kha and Mou. These differences are consistent with previous observations of altitudinal climate gradients in the region (Labhar and Lebaut, 2012).

Effect of the site on needle sclerenchyma cells

Needle sclerenchyma cells are known to contribute to structural integrity and environmental adaptation in conifers. Our results suggest that low-altitude conditions at Tam are associated with increased wall thickness in endodermal cell type A (7.76 ± 3) and in resin canal cell types A' (13.33 ± 5.87), as well as a decrease in the wall thickness

of endodermal cells type B (2.92 ± 2.24) and resin canal cells type B' (5.91 ± 3.99). Compared to the high-altitude sites were respectively, Kha and Mou the wall thickness of type A cells (6.09 ± 3.59 ; 4.22 ± 2.74) and type A' (10.14 ± 6.11 ; 10.27 ± 5.69) is decreased, while type B (3.8 ± 2.40 ; 5.23 ± 2.29) and type B' (8.57 ± 5.33 ; 10.09 ± 5.28) is increased.

This observation indicates that *C. atlantica* exhibits anatomical plasticity in response to environmental gradients. Previous studies in other species (Ye et al., 2020; Kim et al., 2014) suggest that auxin transport, cell differentiation, and stress pathways can mediate leaf and cell shape in response to altitude. Although genetic or molecular pathways (auxin-related genes) are hypothesized mechanisms, not measured in this study, the observed anatomical variation is consistent with environmental regulation of sclerenchyma cells.

These findings are consistent with ecological observations that *C. atlantica* in lowland, warmer, and drier conditions develops sclerenchyma features

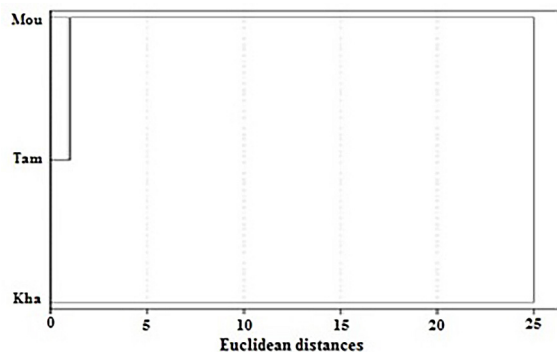


Figure 8. Dendrogram of compared samples of Moudemame (Mou), Tamrabta (Tam) and Kharzouza (Kha) populations constructed on the basis of the shortest Euclidean distances

that may reduce evapotranspiration and increase stress tolerance, supporting the hypothesis that environmental factors influence cell morphology.

Sclerenchyma cell discrimination of cedar populations

The analysis of sclerenchyma cell types in the endoderm and surrounding resin canals using mean number and average frequency revealed that Tam, Kha, and Mou populations can be differentiated based on the frequency of specific cell types. Type A cells were predominant in the Tam population, while Kha and Mou populations showed more balanced frequencies of types A and B. These differences were statistically significant (Figure 5).

Our study demonstrates for the first time that the frequency of sclerenchyma cell types in *C. atlantica* needles can discriminate between natural populations, even when populations are geographically close. This represents a novel anatomical marker for population differentiation, complementing previous taxonomic and environmental studies (Jasińska et al., 2013; Boratyńska et al., 2008). In addition, in the present study, the results support the hypothesis of a clear genetic distinction between the Kha and Tam populations, as established by Terrab et al. (2008). The large F_{ST} distance (0.356) and the limited fragment sharing (66) physically translate into clear structural differentiation in traits A, A', B, and B' (Figure 7) according the Student's t-test ($P \leq 0.01$), thus reinforcing the status of these regions as separate genetic groups. The observed variations in needle traits, which are attributed to the different environmental conditions in the Ifran (Tam) and Azrou (Kha) regions, are consistent with the genetic analysis conducted by Terrab

Table 6. Discrimination power (λ : Lambda of Wilks. P significance of λ) between analyzed characters of needles of populations

Parameter	A	B	C	A'	B'	C'	NCSH	ACSH	NCSE	ACSE	NCSCR	ACSCR
λ	0.713	0.633	0.994	0.657	0.679	0.872	0.806	0.818	0.953	0.805	0.999	0.975
P	0.012*	0.003**	0.924	0.004**	0.006**	0.169	0.061	0.073	0.532	0.060	0.993	0.732

Note: A – sclerenchyma cells with thick walled and restricted lumen in the endoderm; B – sclerenchyma cells with intermediate character in the endoderm; C – sclerenchyma cell with thin wall and wide lumen in the endoderm; A' – sclerenchyma cells with thick walls and restricted lumen surrounding resin canal; B' – sclerenchyma cells with intermediate character surrounding resin canal; C' – sclerenchyma cells with thin wall and wide lumen surrounding resin canal; NCSH – number of sclerenchyma cells at the hypoderm; ACSH – area of sclerenchyma cells at the hypoderm (μm^2); NCSE – number of sclerenchyma cells in the endoderm; ACSE – area of sclerenchyma cells in the endoderm (μm^2); NCSCR – number of sclerenchyma cells surrounding the resin canals; ACSCR – area of sclerenchyma cells surrounding the resin canals (μm^2); Significance at the $P \leq 0.01$ (**) or $P \leq 0.05$ (*) levels.

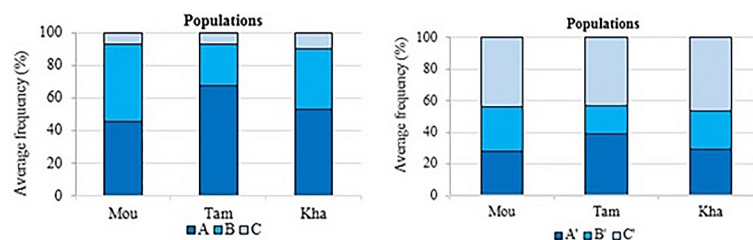


Figure 9. Frequencies of cell types in the endoderm and surrounding resin canals in the three populations

et al. (2008). Similarly, with the study of Wahid et al. (2009) illustrated that the Tamrabta population in the Ifrane region is characterised by a limestone substrate. In parallel, Zine el Abidine et al. (2014) revealed that the most stressed Atlas cedar populations are found in the Ifrane region on a limestone substrate, while the least stressed populations are found in the Azrou region on a basalt substrate.

The observed variation in sclerenchyma cells aligns with patterns found in other conifers and xerophytes, where structural adaptations support leaf stability and water retention under stress (Grill et al., 2003; Kivimäenpää et al., 2003; Adame-González et al., 2019). Thus, the anatomical differences likely reflect both genetic background and environmental modulation, supporting the ecological relevance of these traits (Figure 9).

Synthesis and implications

Overall, our results suggest that:

- *C. atlantica* exhibits population-specific sclerenchyma traits correlated with altitude and climate.
- Type-specific sclerenchyma frequencies can serve as a diagnostic tool for population differentiation.
- Anatomical plasticity may contribute to stress tolerance and survival under varying environmental conditions.

These findings fill a knowledge gap regarding anatomical adaptation in *C. atlantica* and provide new insight for conservation strategies, particularly in the context of climate change. These results support our hypothesis that sclerenchyma cell types respond to climatic gradients and may serve as markers for population differentiation. These results could be crucial for the conservation of the genetic heritage of Moroccan cedars. To verify and clarify this finding, we should plan to grow these populations ex situ over multiple years

to ensure the long-term sustainability of these forests and study the adaptive resilience of the cedar.

Identifying useful markers for the genes that control sclerenchyma cells A, B, A' and B' could help us to manage and conserve the genetic diversity of this increasingly vulnerable species in the face of climate-driven environmental change.

CONCLUSIONS

In summary, this study demonstrates that the frequency of sclerenchyma cell types in needles can effectively discriminate between natural populations of Atlas cedar forests of the Moroccan Middle Atlas, supporting the hypothesis that anatomical traits are moulded by climatic and altitudinal gradients. Specifically, the Tamrabta (Tam) population, located at lower altitude with warmer and drier conditions, is significantly distinguished ($P \leq 0.01$) from the Kharzouza (Kha) and Moude-mame (Mou) populations by higher frequencies of type A (7.76 ± 3) and A' (13.33 ± 5.87) sclerenchyma cells and lower frequencies of types B (2.92 ± 2.24) and B' (5.91 ± 3.99). Kha and Mou populations, both at higher altitudes, exhibit more similar sclerenchyma profiles.

These findings highlight the ecological plasticity of *C. atlantica* and suggest that needle sclerenchyma cell traits are promising morpho-anatomical markers for population differentiation, complementing previous genetic and morphological studies.

The results also indicate that anatomical adaptation of sclerenchyma cells likely contributes to population-specific stress tolerance, particularly under varying temperature and precipitation regimes. This provides insight for conservation planning, by identifying populations that may be more vulnerable to climate change.

Overall, this study demonstrates for the first time that the frequency of sclerenchyma cell types in the endoderm and surrounding resin

canals can discriminate between natural populations of *Cedrus atlantica* in the Middle Atlas of Morocco. The Tamrabta population was found to differ significantly from Kharzouza and Moude-mame populations in the frequencies of types A, B, A', and B' sclerenchyma cells. These results provide a novel anatomical marker for population differentiation and show that needle sclerenchyma traits respond to local climatic and altitudinal conditions, revealing previously unreported patterns of anatomical variation in this species.

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